EFFECT OF SOIL SUCTION ON SOIL MICROBIAL ACTIVITY

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ABSTRACT

In Malaysia, the total oil palm acreage rises from year 1970-2000. Palm oil mill effluent (POME) is a residual liquid waste product obtained after extraction of soil from the fruits of oil palm and has high content of nutrients, total solids, total suspended solids that causes pollution. Palm oil mill generated huge POME with an average of 53 million m3 and is poured away into available land near the mill. By using soil microbial, it is able to treat the contaminated soil that is caused by POME. However, soil bacteria can only survive in an optimum condition, thus, the water content in the soil should be determined. Therefore, two samples of soil, (i) uncontaminated soil (ii) contaminated, are tested with different suctions by using Vapour Equilibrium Technique to find the optimum water content and suction for the soil microbial to survive. In order to check the reduction of contaminants which is Carbon (C) in the contaminated soil, the initial and final total organic carbon (TOC) of contaminated soil is recorded. The identification of the soil bacteria is by using Spread Plate Method. The test is conducted on the uncontaminated soil to identify the fungi and bacteria that digested the carbon in the soil. Based on the results, there is a reduction of the contaminant in the polluted soil by 3.884 mg/L. The optimum water content and suction for the soil bacteria to make it able to reduce the contaminant is 83.49% and 10.58 MPa respectively. Therefore, the soil microbial is able to treat contaminated soil caused by POME with an applied suction of 10.58 MPa and an optimum water content of 83.49%.

ABSTRAK

Di Malaysia, jumlah keluasan kelapa sawit meningkat dari tahun 1970-2000. Efluen kilang minyak kelapa sawit (POME) adalah sisa bahan buangan cecair yang diperolehi selepas pengekstrakan tanah dari buah kelapa sawit dan mempunyai kandungan yang tinggi nutrien, jumlah pepejal, jumlah pepejal terampai yang menyebabkan pencemaran. Kilang minyak kelapa sawit menghasilkan POME yang tinggi dengan purata 53 juta m3 dan dibuang ke dalam tanah yang ada berhampiran kilang. Dengan menggunakan mikrob tanah, ia mampu untuk merawat tanah yang tercemar yang disebabkan oleh POME. Walau bagaimanapun, bakteria tanah hanya boleh hidup dalam keadaan yang optimum, dengan itu, kandungan air di dalam tanah hendaklah ditentukan. Oleh itu, dua sampel tanah, (i) tanah yang tidak tercemar (ii) tercemar, diuji dengan sedutan air yang berbeza dengan menggunakan teknik wap keseimbangan untuk mencari kandungan air yang optimum dan daya sedutan untuk mikrob tanah untuk terus hidup. Dalam usaha untuk memeriksa pengurangan bahan cemar dimana ia adalah Karbon (C) di dalam tanah, jumlah karbon organik (TOC) dalam tanah yang tercemar yang awal dan akhir ditentukan. Pengenalpastian bakteria tanah adalah dengan menggunakan Kaedah Penyebaran Plat. Ujian ini dijalankan di atas tanah yang tidak tercemar untuk mengenal pasti kulat dan bakteria yang dicerna karbon di dalam tanah. Berdasarkan kepada keputusan, terdapat pengurangan bahan cemar di dalam tanah yang tercemar oleh 3,884 mg / L. Kandungan air yang optimum dan daya sedutan yang optimum untuk bakteria tanah mampu mengurangkan pencemaran adalah 83,49% dan 10,58 MPa masing-masing. Oleh kerana itu, mikrob tanah mampu merawat tanah yang tercemar yang disebabkan oleh POME dengan meletakkan daya sedutan 10.58 MPa dan kandungan air optimum 83.49% dalam tanah.

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NA	Nutrient Agar
PDA	Potato Dextrose Agar
SWCC	Soil-Water Characteristic Curve
TOC	Total Organic Carbon

Chapter 1

INTRODUCTION

1.1 INTRODUCTION

Palm oil is one of the most important vegetable oils in the world's oil and fats market. The development of oil palm in Malaysia has been acknowledged by many. In 1920, the hectarage of palm oil plantation is 400 and it increases to 5,000,109 hectares in 2011. Palm oil mill generated huge POME (Rupani, *et al*., 2010) with an average of 53 million m3 (Madaki and Seng, 2013). A rapid increase in both downstream and upstream activities imposed an impact on environment.

Wastewater is discharged in the process of extracting the oil from the palm oil. This wastewater is called as palm oil mill effluent (POME). POME is a residual liquid waste product obtained after extraction of oil from the fruits of oil palm and has high content of nutrients, total solids, total suspended solids that causes pollution (Rupani, *et al*., 2010). POME is disposed into available land, waterways or non-concrete lining pond and cause pollution to the soil, ground water and surrounding environment.

The capability of POME to pollute soil and groundwater is due to soil suction that is induced in the soil. Soil suction is an ability of a soil to store water. According to Murray and Sivakumar (2010), soil suction is an ability of a soil to absorb additional water, whether it is fully saturated or unsaturated.

However, polluted soil can be cleaned up by the use of microorganisms that is available in soil. In soils, there are numerous living organisms which includes bacteria, fungi, algae, protozoa and viruses. These organisms live together as one community and very important for passive and aggressive treatment in subsurface regime (Yong, *et al*., 2007). There are many researchers have done in remediating soil by using soil microbes such as Ojonoma and Udeme (2014).

1.2 PROBLEM STATEMENT

Palm oil mills has generated to extremely high polluting waste-water, known as Palm Oil Mill Effluent (POME). POME that is produced due to the processing of palm oil is often discarded to an unlined concrete pond. Due to soil suction, this problem leads to the leaching of contaminants that pollute the soil and groundwater. Apart from that, POME releases methane gas to atmosphere. POME has extremely high content of degradable organic matter (carbon) and nutrients such as nitrogen, phosphorus and calcium (Ojonoma & Udeme, 2014). POME also contains a very high biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which is 100 times more than the municipal sewage.

POME is a not a toxic material, since there is no chemical added during the oil extraction process. However, it is reported that POME has caused environmental issues due to the reduction of oxygen. These oxygen is needed by aquatic life, unfortunately, the depletion of oxygen is due to the organic and nutrient contents.

Due to high contents of nutrients in POME it is necessary to treat the POME infiltrated into the ground. Literature suggested that, soil microbe could be beneficial in treating the POME contaminated soils. This study is conducted to identify the optimum suction for microbial activity to take place as well as to determine the effectiveness of soil microbe in treating the contaminated soil.

1.3 OBJECTIVE

The main objective of this study:

a) To obtain the optimum suction and water content of a soil for soil microbial to survive.
b) To identify the type of microbe present in soil.
c) To check the effectiveness of soil microbe to treat the POME contaminated soil.

1.4 SCOPE OF STUDY

The main purpose of this research is to obtain optimum suction and water content of a soil for soil microbial to survive, to identify the type of microbe that is available in soil and lastly is to check the effectiveness of soil to treat POME contaminated soil.

The test will be conducted in three laboratory, known as, geotechnical laboratory, environmental laboratory and biotechnology laboratory. The soil and POME which is obtained from palm oil mill in Jabor, Pahang are considered in this study. The samples are generically produced to imitate POME contaminated soil.

An establishment of soil-water characteristic curve is to be studied in order to achieve the first objective which is obtaining optimum suction and water content for the soil microbial to survive. The establishment is done with drying test by using vapour equilibrium technique to induce suction in soil in the range from 3 MPa to 300 MPa. Only one contaminant is considered, namely, Carbon (C) to assess the ability of soil microbe to treat the POME contaminated soil.

Chapter 2

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter presents a brief reviews on palm oil mill effluent (POME), soil suction, soil-water characteristic curve (SWCC) and the application of soil microbiology. In addition, few techniques to control suction and measure suction is comprehensively explained in this chapter such as axis translation technique, osmotic technique, vapour equilibrium technique and chilled mirror dew point technique.

2.2 PALM OIL MILL EFFLUENT (POME)

In Malaysia, the acreage of palm oil has increased from 320 to 3,338 hectares from the year 1970 to 2000 (Rupani, *et al*., 2010). According to Reeves *et al*. (1999), palm oil is an edible plant oil derived from the pulp of oil palm *Elaeis gyuineensis* (Ibe, *et al*., 2014). Table 2.1 shows the agriculture acreage in Malaysia from year 1970 to 2000.

Table 2.1 Malaysia – Agriculture Acreage 1970-2000 [1000 Hectares] (Rupani, *et al*., 2010)

Crops	**1970**	**1985**	**1990**	**1995**	**2000**
Oil palm	320	1,482	2,030	2,540	3,338
Rubber	2,182	1,949	1,837	1,679	1,590
Crops	1970	1985	1990	1995	2000
Rice	533	655	681	673	692
Coconut	349	334	316	249	116

Palm oil is extracted by using huge amount of water. According to Yacob *et al*. (2005), palm oil mills in Malaysia in 2004 generated an average of 30 million tonnes of POME while, Variappan and Yen (2008) recorded that 66.8 million tonnes of POME were produced in 2005 (Madaki & Seng, 2013). The production of POME is increasing over the years due to the high growth rate of palm oil production.

2.2.1 Composition of POME

Based on Madaki and Seng (2013), POME is a thick brownish colloidal mixture of water, oil and fine suspended solids when it is fresh. POME consists of water, cellulosic material, fat, oil, suspended solids and total dissolved solids (Rupani, *et al*., 2010) and very high degradable organic matter (Ojonoma & Udeme, 2014). The characterisitics of POME is said to be relied upon the quality of the raw material and the production process of palm oil. POME also possessed 4.5 pH value due to the existence of organic acids and contains very high Biochemical Oxygen Demand (BOD).

Solids that is found in POME known as palm oil mill sludges (POMS) are leaves, seed shells, decanter cake, fibre and empty fruit branches. These solids comprises in POME in the range of 18,000 mg $L^{-}1$ up tp 40,000 mg $L^{-}1$ as shown in Table 2.2.

Table: 2.2 Characterisic of raw POME and the regulatory discharge limits (Rupani, *et al*. ,2010)

Parameters *	**Value**	**Regulatory discharge limits**
Temperature	80-90	45
pH	4.7	5.0 – 9.0
Biochemical Oxygen Demand	25,000	100 (50)
Chemical Oxygen Demand	50,000	-
Total Solids (T.S)	40,500	-
Total Suspended Solids (T.S.S)	18,000	400
Total Volatile Solids (T.V.S)	34,000	-
Oil and Grease (O&G)	4,000	50
Ammonia-Nitrate (NH3-N)	35	150
Total Kjeldahl nitrogen (TKN)	750	200

*All values are in mg/L except for pH

2.2.2 POME as pollutant

POME has been reported as a source of pollutant to the soil and groundwater as it could alter the physicochemical properties of soil, reduce the growth of oil palm seedlings, oxygen depletion in water body and disturb the bio-mass in POME polluted soil (Ojonoma & Udeme, 2014). The high concentration of BOD in raw POME is caused by the residual oil. Before the oil can be completely decomposed, the microbes in water require or demand high level of dissolved oxygen. These microbes take dissolved oxygen faster than atmospheric oxygen can dissolve in water in order to digest the organic matter. Hence, these phenomenon could result in oxygen depletion in water way as well as the death and reduction of aquatic life. Bek Nielsen, *et al*. (1999) found out that POME has caused problem in water quality (Okwute, *et al*., 2007).

According to Orji, *et al* (2006), the pollution due to palm oil has become a serious problem because of the rapid growing rate of the palm oil mill industries (Ibe, *et* *al*., 2014). POME is normally discharged on farmland (Ibe, *et al*., 2014) near the mills. In Malaysia, the most recognised method to treat POME is utilised by open pond system due to its low capital and operating cost (Baharuddin, *et al*., 2010) that will cause the effluent to seep into the ground, therefore, the pollution will occur.

2.3 SOIL SUCTION

Soil suction is defined by Richards (1974) as the water potential in a soil-water system. It is the state of soil water (Fredlund & Rahardjo, 1993) or ability of a soil to absorb additional water, whether it is fully saturated or unsaturated (Murray & Sivakumar, 2010). The free energy of the soil water can be measured in terms of the partial vapour pressure of the soil water (Fredlund & Rahardjo, 1993).

Soil suction shows a relation with relative humidity(%). According to (Fredlund & Rahardjo, 1993), when the relative humidity is equal to 100% in a soil, it would indicate the suction in the soil, (i.e soil suction = 0). However, if the relative humidity value is less than 100%, then there is a presence of suction in the soil. It is useful to know the relationship between the suction value of a soil and its water content in order to know the behaviour of volume changes and shear strength in the soil (Barbour, 1998).

Total suction is calculated as:

$$h_t = h_m + h_\pi \qquad \text{Eq. 2.1}$$

where h_t is the total suction of the soil, h_m is the matric suction, and h_π is the osmotic suction.

Total suction can be written using Kelvin's equation associated with relative humidity:

$$h_t = \frac{RT}{V}\ln\left(\frac{P}{P_o}\right) \qquad \text{Eq. 2.2}$$

where h_t is total suction, R is universal gas constant, T is absolute temperature, V is molecular volume of water, P / P_o is relative humidity, P is partial pressure of pore water vapor, and P_o is saturation pressure of water vapor over a flat surface of pure water at the same temperature.

Due to soil suction that occurs in soil, POME which is mainly constitutes of 97% of water could infiltrate the soil and inevitably seep into the ground and eventually contaminate groundwater as described by Bek Nielsen (1999).

2.4 SUCTION CONTROL TECHNIQUES

Several techniques have been performed in controlling suction of soils such as axis translation technique, osmotic technique, vapour equilibrium technique and chilled mirror dew point technique. Each technique induces different suction values as well as each of it has its own unique way to implement the test.

2.4.1 Axis Translation Technique

This technique has been used by many researchers and only allowed for a measuring a matric suction in the soils (Fredlund & Rahardjo, 1993). The soil specimen is placed on top of a saturated high entry disk in an air pressure chamber. According to Olson and Langfender (1965), in order to make sure that there is a good contact between soil specimen and the disk, a 1kg mass is put on top of the soil specimen. The setting of this technique including the soil specimen to be put on the disk has to be performed rapidly of approximately 30s. The water pressure compartment below the disk has to be maintained nearly zero pressure by increasing the air pressure in the chamber. The pressure transducer connected to the water is used as null indicator.

The matric suction of the soil specimen is measured by the difference between the air pressure and negative water pressure ($u_a - u_w$) in kPa at equilibrium (Fredlund & Rahardjo, 1993). There is signification relationship between the decreasing of water content and increasing of matric suction (Fredlund & Rahardjo, 1993).

Generally, axis translation technique can be used to measure negative pore-water pressure wth a maximum high air entry disk value up to 1500 kPa (Fredlund & Rahardjo, 1993).

2.4.2 Osmotic Technique

Osmotic technique is developed by a biologist then adopted by soil scientists and geotechnical researchers due to limitations in axis-translation technique (Ng & Menzies, 2007). Osmotic technique control matric suction of a soil (Delage, *et al*., 2008). The soil specimen is placed in contact with a semi-permeable membrane behind an aqueous solution containing large sized polyethyleneglycol (PEG) molecules that is circulated. The membrane is permeable to water and ions in the soil but it is not permeable to large solute molecules and soil particles (Ng & Menzies, 2007). PEG molecules cannot pass through the membrane while water molecules can, an osmotic suction is applied to the sample through the membrane. According to Blatz, *et al*. (2008), suction value can obtained in the range of 0 to 10 MPa.

The limitation of this technique is the membrane is sensitive to microbial attack. Due to microbial attack, the PEG solution can be easily infiltrated into the membrane and suction is no longer controlled (Delage, *et al*., 2008). Therefore, it is important to use penicillin in the solution before starting the test in order to prevent from bacteria attack.

2.4.3 Vapour Equilibrium Technique

Vapour equilibrium technique is done by controlling the relative humidity in a closed system (Delage, *et al*., 2008). In this technique, soil specimens is placed on top of a porous disk over a salt solution in a glass desiccator (see Figure 2.1) and generally, the time taken for the soil to achieve an equilibrium state is observed (Tang & Cui, 2005).

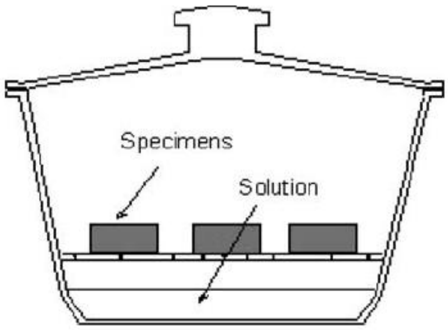

Figure 2.1: Vapour equilibrium technique

Suction is produced in a soil specimen due to an event of a net water exchange between liquid and vapour phases in a desiccator (Delage, *et al*., 2008). The net water exchange between liquid and vapour phases has to be at equilibrium in order for the suction value to be taken. However, the primary disadvantage in this technique is that the time to reach moisture equilisation is very long (two to three months) because of the vapour transfer depends on the diffusion process that controls the transfer of water vapour (Blatz, *et al*., 2008). Secondly, a small changes in temperature will effect the soil suction, therefore there should be a very tight control on temperature (Blatz, *et al*., 2008).

Two types of osmotic solution used in this technique which is (1) saturated salt solutions and (2) unsaturated acid solutions (Blatz, *et al*., 2008). The advantage of using saturated salt solutions is that the concentration of osmotic solutions does not vary though there is an exchange of water between soil specimen and vapour environment and due to safety concern (Blatz, *et al*., 2008). The drawbacks of using salt solution is that each type of salt will control the suction in the specimen based on the purity and specific behaviour and properties of the chemical component (Blatz, *et al*., 2008). The range of suction in vapour equilibrium technique ranging from 3 MPa to 1000 MPa (Ng & Menzies, 2007). The second osmotic solution which is the unsaturated acid solution, it imposed a higher suction than the saturated salt solution. However, the concentration of the solution varies in the event of water exchange, thus, it effects the targeted suction value.

2.4.4 Chilled-Mirror Dew-Point Technique

Chilled-mirror sensing technology has been used since the 1950s for determination of dew-point temperature in a closed, humid environment (Juca, *et al*., 2004). This approach allows the total suction to be determined with an upper limit of 60 MPa with an equilibrium time of around five minutes (Murray & Sivakumar, 2010). Humidity measurement involves thermoelectric chilling for a reflective surface, usually a metallic mirror, to a temperature at which condensation from ambient water vapour in the closed chamber forms on the mirror surface (Juca, *et al*., 2004). A beam of light, typically from a light-emitting diode (LED), is directed to the mirror and reflected to a photodetector (Juca, *et al*., 2004). When condensation occurs as the mirror is cooled to the dew-point temperature, the light reflected from the mirror is scattered and the intensity detected by the photodetector is consequently reduced (Murray & Sivakumar, 2010). The dew-point temperature is maintained contant by a microprocessor circuit and measured by a resistance thermometer embedded in the mirror. The total suction can be calculated using Kelvin's law.

2.5 SOIL – WATER CHARACTERISTIC CURVE (SWCC)

SWCC is discussed because there is a dinstinctive relationship between water content and soil suction. There are many terms used to refer the relationship between soil suction and water content. For instance, soil-water characteristic curve, water-suction relationship, retention curves, and moisture retention curves.

However, the term "soil-water characteristic curve" is used to describe the water content of the soil that is measured. The term characteristic is used to explain the character or behaviour of the unsaturated soil such as the permeability functions, water storage functions, shear strength functions and thermal property functions (Fredlund, *et al*., 2011). SWCC is identified as the key soil information required for the analysis of seepage, shear strength, and volume change problems involving unsaturated soils (Juca, *et al*., 2004).

2.6 MICROORGANISMS IN SOILS

Biological properties of soils are very important factors in the passive and aggressive treatment and management of organic chemical in the subsurface soil regime (Yong, *et al*., 2007). According to Lipman (1931), Microorganisms that live in soil consist of bacteria, ray fungi, algae, protozoa, round worms, Rotarians, and larvae of insects. These microorganisms are very small and cannot be seen by naked eye except for certain type of fungi, and insects. Microorganisms in soils find soil as their medium in performing their activities such as degradation of organic matter, disease suppression, disease and nutrient (Jenkins, 2005). Apart from that, microorganisms obtain an enough supply of energy and nutrient from soil for their growth and production.

2.6.1 Bacteria

Bacteria are the most ample microorganisms in soil. And it has a size range of 5 to 8 microns (Lipman, 1931). Due to its size, the quantity of bacteria in soil per unit area is numerous. Bacteria activity is affected by the presence of oxygen, moisture, inorganic compound, or any change in temperature of the surrounding (Lipman, 1931). Soil bacteria is allocated into three groups in which the first group is cocci, second is bacilli and the third is spirilla.

The optimum temperature for most of the bacteria to grow is in between 20 to 30^{o}c (Jenkins, 2005). The sufficient amount of moisture and oxygen is required for the bacteria to survive. There are numerous processes brought by soil bacteria in order for it to survive if any lack of sources. These organisms is mutually supporting with one another.

2.6.2 Fungi

Fungi is a microscopic plant-like cell that grows as long strands called as hyphae (Hoorman, 2011). Fungi acts as a recycle bin and it reabsorbs nutrients in soil (Yong, *et al*., 2007). The size of fungi is 0.001 inch in diameter (Hoorman, 2011). Mushroom is one type of all fungi and its features are spores, gills and fruiting bodies. Due to its size, fungi dominates the soil biomass although it has lower number of individuals in healthy soils (Hoorman, 2011). Fungi grow more slowly in acidic pH range than bacteria and it is sensitive to changes in moisture levels (Yong, *et al*., 2007).

Fungi plays a very important role in improving the soil structure and increasing nutrient uptakes (Hoorman, 2011). Fungi also acts as decomposer in soil web which the soil web is meant by the community of organisms that live in the soil and how it interacts with environment, plants and animals (Lipman, 1931). Soil is dominated by a population of fungi although its number is lesser than the number of bacteria (Jenkins, 2005). Fungi has the ability to store and recycle carbon (C) because it is a carbon use efficiency of 40-50% and it is also noticed that fungi consists of much higher of carbon (C) content than nitrogen (N) in their cells compared to bacteria (Hoorman, 2011). Many plants develop and use a certain types of fungus and bacteria to raise up the nutrient extraction from soil (Hoorman, 2011).

2.6.3 Microorganism as an agent in treating contaminated soil

Bacteria are becoming increasingly important in bioremediation. Bacteria are capable of filtering and degrading a large variety of human-made pollutants in the soil and groundwater. Case studies showed that microorganisms resided in cow dung & chicken droppings is able to reduce levels of POME in soil (Ojonoma & Udeme, 2014). The microorganisms that present is cow dung is of the genera *Pseudomonas* (Ojonoma & Udeme, 2014). *Pseudomonas aeruginosa* is bacteria that is able to utilize oil as carbon source & could be useful in bioremediation of highly contaminated soil (Das & Mukherji, 2013).

Other than bacteria, fungi are also the choice in bioremediation. Fungi are known to degrade hydrocarbon (Omokaro, 2009). According to Obire (1988), there are several species oil-degrading fungi found in petroleum-producing regions of Nigeria, namely, *Aspergillus niger*, *Penicillium glabrum* and *Trichoderma harzianum* (Omokaro, 2009). While, *Paecilomyces inflatus* is relevant in degrading lignocelluloses in nature, especially soil (Beata, 2007)